

Figure 1:

Amino acid sequences of Cpn60 and Cpn10:

SEQ ID No 1: Cpn10 (encoded by nucleotides pos. 458-751 of Figure 2):

MKIRPLHDRVRRKEETATAGGIILPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 2: Cpn60 (encoded by nucleotides pos. 800-2446 of Figure 2):

MAAKDVLFGDSARAKMLVGVNILADAVRVTLGPKGRNVVIEKSFGAPIITKDGVSV
REIELKDKFENMGAQMVKEVASQANDQAGDGTATTATVLAQAIIEGLKSVAAGMNP
MDLKRIGDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADEVGRLIAEAMEKVG
KEGVITVEEGKGLEDELVDVEGMQFDRGYLSPYFINNQEKMTVEMENPLILLVDK
DNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVAAVKAPGFGDR
RKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEASV
NTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVDDA
LHATRAAVEEGVVAGGGVALIRALSSVTTVGDNEDQNVGIALALRAMEAPIRQIAGN
AGAEGSVVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPKAVTRSSLQAAASIAGL
MITTEAMVADAPVEEGAGGMPDMGGMGGMGMPGMM

Figure 2:

SEQ ID No 3: DNA coding for Cpn60 and Cpn10:

Cpn10, pos. 458-751

Cpn60, pos. 800-2446

atcaaaaaatgcagcaaggacagattcctgccaagaattagcagaagggttcttgtagcactggccggcgcttattattaacgccgg
gtttgtcactgatgcgctgggtttacattactcgtccccgcgacgcgtaaaagcgttggtccataaggtagtgcatttattaccctc
gcatgatgactgcaagcagcttcaagcgacgggtagtttcaggaaggctcgtttaaagatgtacattcgacactgactcgcaaagca
gtcatgaaaaaatcacaattgaaggcgaatataccaagacgataagtaggtatttttcggctagccggtgaaatcctagtaaaagccc

cgataaattaaccatctatttttcacagaggcaatttagcctttgtttaccttattgatcctaatacttgggatccaacagttggagagtctagc
aaatgaaaatccgtccattacatgatcgtattgtgttcgccgtaagaagaagagaccgcaactgcgggtggtatttttacc
ggcgctgcggcagaaaaaccaaataaggtgtgttatctgtgggtactggcgtattcttgataatggttcagtgaagcgctggc
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aagtgatatctacggcggttttagaagcttaattattacactcacttttttatttaacctacaaaatttaaggaaagatcatggctgctaaagacg
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aattcgaaaacatggcgccacagatggttaaggaagttgcttctcaagccaacgaccaagccggtgacggcacaacgacagcgactg
tactagcacaggcgattatcagcgaaggcttgaatctgttgcggctggcatgaatccaatggatcttaaacgtggattgataaagctac
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ctagggtgctttagaaactgcggatccttcttgggttacggcaagcagggtgttatcgataaagaaaacaccgtgattgttga
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gaccgtgttgacgatgcacttcatgcaactcgcgcagcggttgaagaaggtgttgttgcgggtggtggtgttgccttgatcgcgcactct
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atggcgatatgattgcgatgggtatttttagacctgcaaaagtcacgcgttcatctctacaagccgcggcgtctatcgaggtttgatgat
cacaaccgaagccatggttgcggatgcgcctgttgaagaaggcgtggtggtatgcctgatatggcgggcatgggtggaatggcggg
tatgcctggcatgatgtaatcatttgcattgtcctgatctgcttaccgtgtaaaaagatcaggctcaaggctgtctctataaaaagcc
gtatctttgatgagtgttgccttctgtgaaaacgacattcttgagtgccgcttttttattttggtcataaaattcagaatattgtgtaatttta
tgtaactagctggcctataatgttgagttcctctgggtggcatgatctcatggtacttcaacttaagcctgattcactgcg
gccttaacagtaaaaataaacgcaacgtagaacaataaagcgtatggcattaatgaagacggctgcatttaattcagatc

Figure 3:

SEQ ID No 4: Amino acid sequence of esterase cloned from *Oleispira antarctica* (EstRB8):

EstRB8 (encoded by nucleotides 1145 to 2143 Frame 2 of Figure 4) 333 aa

MKNTLKSSSRFSLKQLGTGALISSLFFGGCTTTQQDNLYTGVM SLARDSAGLEVKTA
SAGDVNLTYMERQGSDDNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG
HGDSEQLLTDDYGLIKQAERLDIFLSGLGVNSFHIAGNSMGG AISAIYSLSHPEKVKSL
TLIDAAGVDGDTESEYYKVLAEKGK NPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL
RKT LARAEINN KIFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAA AFKKIIPQATVHIFPEVGHLPMVEIPSESAKVYEEFLSSIK

Figure 4:

SEQ ID No 5: DNA fragment from plasmid pBK1Est coding for esterase of *Oleispira antarctica* (EstRB8):

Nucleotide positions 1-100 correspond to reverse complement of positions 1196-1121 and 3799-3939 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene).

Positions 101-105 are *Bam*HI – *Sau*3A1 fusion and positions 3795-3798 are *Sau*3A1-*Bam*HI-fusion.

acaggaaacagctatgaccttgattacgccaagctcgaaattaaccctcactaaaggaacaaaagctggagctcgcgcgcctgcag
gtcgacactagtggatcaacggcgttcattggtactggctgagtcagcgtcataatgccgatgcgatactggcgcgtcattgactgactgag
tctctgctagcaccgatttttctaatagcgcagcttcttttattctgaacgggcaactgatgtagtttttactaaccggccttttaggcatgg
taaactcttcgataattcaaaattattactgttcattacaatcatagtagcaggctagaggcccaaaattgcagctgatattcacctttattatc
taagcattattacactcatcgcgggtgttattaattgtgctaaataaaaatacccgtagcggaaaaattcagcaaatagccaaagaaaacga
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ctcattcaagcaactgacgaacactatggcctagcgtttaagacctttgactgtcgagcgttagaaccttcagggtattcacaatagcagcttt
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gtcaccaacaatcaatcaaacaccaataccaatcgcaaaaactcataaaactagccgatcaccaatcccaaaagcgttcaaaaatgaa
acgagcacgtcacacaaaatcaatttatacgtacgaaccagggtcaaaactatcgttttttgagcacgtttgtccactaatgaaagaga
aaagtcgttaattcactggccttttggcgtatccgcaccttcacatagaaattagtaattggcatgctactggcctttaaaaagaatcagtttaatt
gaagaaacctcgttatctcagccattaccgctgtagccgaatttgcgcttatcctcagccatgattaaactgacgccaatfaataagac
atactaattaataactcccttaattgagaagaataatgaaaacacactcaaatcctcatcacgttttagtctgaaacaactcggcaccggc
gctctgattatctccagtttgttctcgggtgtgcaccacaacacaagaataattatacacaggggttatgtctcttgcgagagacagc
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SN 10/575,505
Replacement Sheet

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ggttaactcatttcacatcgccgtaattcaatggggggggctatcagcgcaatctacagtttgagtcaccagagaaaagttaaaagtctt
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gatgaagcaagtttgaataccgcatgggtttccatgactcagcctcctttctaccttggccactaagaccttctttattacgtaaaacg
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ccaaattattcaacgaccaagctctgcggtaaaatcgcatgtgggttcttgttttcatcaacagcaacaaacgtgaataccccgtaatcg
cattttctgattatcaaaatacatactttccaccagcatattaacttcaactttfaaactcgtccgccctacctctataacactggcagtcgaatt
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gtgataatattcatcttgccatgagagcttatcatgaagcctgtgcttaaaatcaatcattatatttattcatctttaattgaaataataccaat
atatttcatatataatttcacactaccttatctcactagacttcccgcgataggcgcaaacaaatcaacgcaagttcacaataaagcgggtc
gctgcaacacatgccctagcgtctaaagtagcacgcacaacactggccagtcgtactagcccccttgcgattcgtgcagacgagcaac
aagcgctattaaacttacctaaatttctaaccaccaccattgggtctttccacaaaactcaaaaaactcgtaaatccgcttgcaatttaaacg
cgatgacatagatctaactgattatcaaaccgcattcaagcgtcattaaaaacgcaccactggcaagaagttctacctgcactgacca
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caatcaataaaaaacgagttattgaggattttaattttaaaacaggtatattaataccctctctcgtagtaaacatgactgtatttacaaaa
ataaatagaggataccatgtcaaacatctggttgaagtaccaaagattgaagtattaaaccgtcaaatggaaaatactgcctgcagcaa
cttaggcattcaaattacagaaattggcgatgattatatcactggcacaatgccagcagatgcacgtaccttcagccaatgggactgatt
catggcggctcaaatgtattgtctggcagaaacactgggcagcatggcagctaactgctgtatttaattgtctcaagaatattgtgttggcc
aagaaattaacgccaaccacatacgcggtgttcgttccggcatagtactggcacagcaacgctagtacacaaaggaagaacctccca
gatttgggaaattcgcatcgttaacgatccaaagaattcaaaaagcttctcgagagtacttctagagcgggccgcccgcatttcc
accgggtgggggtaccaggttaagtgtacccaattcgcctatagtgagtcgtattacaattcactggccgtcgttttac

Figure 5:

Amino acid sequences expressed from vector pBK1CpnEst: - the co-expression of fragments encoding native chaperonines with the esterase gene (EstRB8), all from *Oleispira antarctica*

SEQ ID No 6: cpn10 (nucleotides 113 to 403: Frame 2 of Figure 6) 97 aa:

MKIRPLHDRVRRKEEETATAGGILPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEG DVVVF GKYS GQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 7: cpn60 (nucleotides 455 to 2098: Frame 2 of Figure 6) 548 aa:

MAAKDVLFGDSARAKMLVGVN ILADAVRVT LGPKGRNVVIEKSFGAPIITKDGVSV A
REIELKDKFENMGAQM VKEVASQANDQAGDGT TATVLAQAIISEGLKSVAAGMNP
MDLKR GIDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANA DETVGR LIAEAMEKVG
KEGVITVEEGKGLEDEL DVVEGMQFDRGYLSPYFINNQE KMTVEMENPLILLVDKKI
DNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVA AVKAPGFGDR
RKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEASV
NTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVDDA
LHATRAAVEEGVVAGGGVALIRALSSVTTVVGDNEDQNVGIALALRAMEAPIRQIAGN
AGAEGSVVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPK VTRSSLQAAASIAGL
MITTEAMVADAPVEEGAGGMPDMGGMGGMGGMGMPGMM

SEQ ID No 8: estRB8 (nucleotides 2579 to 3577: Frame 2 of Figure 6) 333 aa:

MKNTLKSSSRFSLKQLGTGALISSLFFGGCTTTQQDNLYTGVM SLARDSAGLEVKTA
SAGDVNLTYMERQGS DKDNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG
HGDSEQLLT TDYGLIKQAERLDIFLSGLGVNSFH IAGNSMGG AISAIYSLSHPEKVKSL
TLIDAAGVDG DTESEYYKVLAEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL
RKTLARAEINN KIFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAA AFKKIIPQATVHIFPEVGH LPMVEIPSESAKVYEEFLSSIK

Figure 6:

SEQ ID No 9: pBK1CpnEst: - the fusion of native chaperonine-coding fragments with
esterase of *Oleispira antarctica* (EstRB8)

The DNA fragment coding for Cpn10 and Cpn60 is flanked by *SacI* site (pos. 69-75) and *SalI* site (encoded by pos. 2138-2143 of Figure 7):

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene)

Small letters – the Cpn10-Cpn60 encoding fragment,

Capital italics – fragments of vector pBK-CMV

Capital letters – fragment coding for EstRB8 from plasmid pBK1Est

ACAGGAAACAGCTATGACCTTGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGGA
*ACAAAAGCTGGAGCTC*cctaacttgggatccaacagttggagagtctagcaaatgaaaatccgtccattacatgatcgatt
gttcttcgccgtaaagaagaagagaccgcaactgcgggtggtatttttaccgggcgtgcggcagaaaaacaaatcaagtggtg
tatctctgtgggtactggccgtattcttgataatggtcagtgcaagcgtggcggtaacgaaggcgatgtgtcgttttgtaaatactc
aggtcaaaatactatcgatcgatggtaagaattattgattgaatgaaagtatatctacggcgttttagaagcttaattattacactca
ctttttatttaacctacaaaatttaaggaaagatcatggctgctaaagacgtattatttggatagcgcacgcgcaaaaatgttgtaggt
gtaaacatttttagccgacgcagtaagagttaccttaggacctaaaggtcgtaacgttggtatagaaaaatcatttgggtgcaccgatcatcac
caaagatggtgtttctgttcgcgtgaaatcgaattgaagacaaatcgaaaacatgggcgcacagatggttaagggaagttgcttca
agccaacgaccaagccggtgacggcacaacgacagcgactgtactagcacaggcgattatcagcgaaggcttgaatctgttgcgg
ctggcatgaatccaatggatcttaaacgtggtattgataaagctacggctgctgtgttgcggccattaaagaacaagctcagccttgcttg
gatacaaaagcaatcgctcaggtagggacaatctctgccaatgccgatgaaacgggttggtcgtttaattgctgaagcgatggaaaaagt
cggtaaagaaggtgtgattaccgttgaagaaggcaaaggccttgaagacgagcttgatgtttagaaggcatgcagttcgatcgcggtt
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aacattggtagtaaacacttgcgcggcacattcaaggttcagcgggttaaagcccctgggttggcgatcgctgtaaacgatgttgca
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Replacement Sheet

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gcacttcgtgcgatggaagctcctatccgtcaaatcgcggtaacgcaggtgctgaagggtcagtggtgttgataaagtgaatctgg
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tatgcctgatatgggcgcatgggtggaatgggcggtatgcctggcatgatgaatcactttgtgattcattgtcctgatctgcttaccgtG
TCGACATATTCAAGATAAAGATGCCTTCACTGACATCAGTCACCAACAATCAATC
AAACACCAATACCAATCGCAAAAACCTCATAAAAACCTAGCCGATCACCAAAATCCCA
AAAGCGTTCAAAAATGAAACGAGCACGTCACACAAAATCAATTTATACGCTAAC
GAACCAGGTCAAACCTTATCGTTTTTTTGAGCACGTTTGTTCCTACTAATGAAAGAG
AAAAGTCGTTAATTCCTGACTGCTTTTGGCGTATCCGCACCTTCACATAGAAATTAGT
AATGGCATGCTACTGGCCTTTAAAAAGAATCAGTTAATTGAAGAAACCTCGCTTA
TCTCAGCCATTACCGCTGTAGCCGAATTTGCGCTTATCCTCAGCCATGATTAACT
GACGCCAATTAATATAAGACATACTAATTAATAACTCCCTTAATTGAGAAGAATA
ATGAAAAACACACTCAAATCCTCATCACGTTTTAGTCTGAAACAACTCGGCACCG
GCGCTCTGATTATCTCCAGTTTGTCTTCGGTGGTTGCACCACAACACAACAGAT
AATTTATACACAGGGGTATGTCTCTTGCGAGAGACAGCGCTGGCCTAGAAGTTA
AAACAGCCTCTGCCGGTGACGTCAATCTTACTTATATGGAACGCCAAGGCAGTGA
CAAAGATAATGCCGAAAGCGTTATTTTATTACACGGTTTCTCTGCTGATAAAGAT
AACTGGATTCTTTTTACCAAAGAATTCGATGAAAAATATCATGTTATCGCTGTCGA
TTTAGCGGGACATGGCGATTGAGAACAATTATTAACGACTGATTACGGTCTCATA
AAACAAGCCGAGCGTTTAGATATCTTCTTATCTGGCTTAGGGGTAACTCATTTC
CATCGCCGGTAATTCAATGGGGGGGGCTATCAGCGCAATCTACAGTTTGAGTCAC
CCAGAGAAAGTTAAAAGTCTTACATTGATCGATGCAGCAGGTGTCGATGGCGATA
CTGAAAGCGAATACTACAAAGTTTTGGCAGAAGGTAAGAATCCTTTAATTGCAAC
TGATGAAGCAAGTTTTGAATACCGCATGGGTTCACCATGACTCAGCCTCCTTTCC
TACCTTGGCCACTAAGACCTTCTTTATTACGTAAAACGCTAGCCCGTGCCGAGATC
AATAACAAAATTTTTTCCGATATGCTGAAAACCAAAGAACGTTTAGGAATGACTA
ACTTTCAACAGAAAATTGAAGTGAAAATGGCTCAACATCCATTGCCAACACTGAT
TATGTGGGGCAAAGAAGATCGCGTTCTTGACGTATCCGCAGCAGCGGCCTTCAAA
AAAATAATTCCACAAGCAACTGTTTCATATTTTTCTGAAGTAGGCCACCTACCTAT
GGTAGAAATTCCTAGTGAAAGCGCTAAAGTTTATGAAGAGTTTTTGTCTCTATTA
AATAAGAGCACATAATCATGACTGACTTATAAACAGCCAAGCATTAAAAATGCTT
GGCTGTTTATTTAATGGCCAAATTATTCAACGACCAAGCTCTGCGGTAAAATCG

CAGTGGGTTTCTTGTTTTTCATCAACAGCAACAAACGTGAAATACCCCGTAATCGC
ATTTTTCTGATTATCAAAATACATACTTTCCACCAGCATATTAAC TTCAACTTTTA
AACTCGTCCGCCCTACCTCTATAACACTGGCAGTCAATTTCGACAATGGTACCTGC
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CGAGAAAAACGAGTCGCTGCAATAAAAGAAACCTCATCCATCCACTGCATTGCA
GTGCCACCGAATAACGTATCATGATGATTTGTTGTCTCTGGAAATACCGCTTTAGA
AATAGTGGTTTTTGATACGCGCTTTCGCTGCGCAATAATATCTTCTCTGCTAAGAG
TTGCGGATGGCATAACATAAACTCGCTTGATTAAGATTAATAATAAATAGTTAACA
GTATATTGAACTGAGGGTCTGAAGAACTCTAATACCTCTGAAGAACTTTGAGGCC
GCTAGAGAGAAAAGACCAGTGATAATATTTTCATCTTGCCATGAGAGCTTATCATG
AAAGCCTGTGCTTAAAATCAATCATTATATTTATTCATCTTTAATTGAAATAATAC
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TAGGCGCAAACAATCAACGCAAGTTCACAATAAAGCGGTTTCGCTGCAACACATG
CCCTAGCGTCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGCCCCTTTGC
GATTCGTGCAGACGAGCAACAAGCGCTATTAAACTTACCTAAATTTCTAACCACC
ACCATTGGTTCTTTTCCACAAACTCAAAAAACTCGTCAAATCCGCTTGCAATTTAA
ACGCGATGACATAGATCTAATCGATTATCAAACCCGCATTCAAGCGCTCATTAAA
AACGCACCACTGGCAAGAAGTTCTACCTGCACTGACCAATATGCAAGCGGCGGC
GGAAGAGCTGCCTTTGATCGATCAAGAAGAAGGGAGCAGCAAAGAGGAAAACA
ATCAAAAAGAGGAGAGCAATCAAATAAAAACGAGTTATTGAGGATTTTAATTTTA
AAACAGGTATATTAATACCCTCTCTCGTAGTAAACAATGACTGTATTTACACAAA
AATAAATAGAGGTATACCATGTCAAACATCTGGTTTGAAGTACCAAAGATTGAAG
TATTAAACCGTCAAATGGAAAATACTGCCTGCAGCAACTTAGGCATTCAAATTAC
AGAAATTGGCGATGATTATATCACTGGCACAATGCCAGCAGATGCACGTACCTTC
CAGCCAATGGGACTGATTCATGGCGGCTCAAATGTATTGCTGGCAGAAACACTGG
GCAGCATGGCAGCTAACTGCTGTATTAATTTGTCTCAAGAATATTGTGTTGGCCA
AGAAATTAACGCCAACACATACGCGGTGTTTCGTTCCGGCATAGTGACTGGCACA
GCAACGCTAGTACACAAAGGAAGAACCTCCCAGATTTGGGAAATTCGCATCGTTA
ACGATCCAAAGAATTCAAAAAGCTTCTCGAGAGTACTTCTAGAGCGGCCGCGGGCCCCA
TCGATTTTCCACCCGGGTGGGGTACCAGGTAAGTGTACCCAATTCGCCCTATAGTGAGT
CGTATTACAATTCCTGGCCGTCGTTTTAC

Figure 7:

Amino acid sequences expressed from vector pBK1CpnSREst: - the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala::est)

SEQ ID No 10: cpn10 (nucleotides 113 to 403: Frame 2 of Figure 8) 97 aa:

MKIRPLHDRVRRKEETATAGGIILPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEG DVVVF GKYSQNTIDIDGEELLILNESDIYGVLEA

Below – ***Capital bold letters*** are the mutations introduced

SEQ ID No 11: stabilized single ring mutant of cpn60 (nucleotides 455 to 2098: Frame 2 of Figure 8) 548 aa:

MAAKDVLFGDSARAKMLVGVN~~IL~~ADAVRVTLGPKGRNVVIEKSFGAPIITKDGVSV
REIELKDKFENMGAQM~~V~~KEVASQANDQAGDGT~~T~~TATVLAQAIISEGLKSVAAGMNP
MDLKR~~G~~IDKATAAVVAAI~~KE~~QAQPCLDTKAIAQVGTISANA~~DE~~TVGRLIAEAMEKVG
KEGVITVEEGKGLEDEL~~D~~VVEGMQFDRGYLSPYFINNQE~~K~~MTVEMENPLILLVDK~~K~~I
DNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVA~~AV~~KAPGFGDR
RKAMLQDLAILTGGQVISEELGMSLETADPSSLG~~T~~ASKVVIDKENTVIVDGAGTEASV
NTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVDDA
LHATRAAVEEGVVAGGGVALIRALSSVTTVVGDNEDQNVGIALALRAMEAPIRQIAGN
AGA~~A~~G~~A~~A~~V~~VVDKVKSGTGSFGFNASTGEYGD~~M~~IAMGILDPK~~V~~TRSSLQAAASIAGL
MITTEAMVADAPVEEGAGGMPDMGGMGGMGMPGMM

SEQ ID No 12: EstRB8 (nucleotides 2579 to 3577: Frame 2 of Figure 8) 333 aa:

MKNTLKSSSRFSLKQLGTGALISSLFFGGCTTTQQDNLYTGVM~~S~~LARDSAGLEVKTA
SAGDVN~~L~~TYMERQGS~~D~~KDNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG
HGDSEQLLT~~D~~YGLIKQAERLDIFLSGLGVNSFHIAGNSMGG~~A~~ISAIYSLSHPEKVKSL
TLIDAAGVDGDTESEYYKVLAEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL

RKTLARAEINNKIFSDMLKTKERLGMTNFQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAAFKKIIPQATVHIFPEVGHLPMVEIPSESAKVYEEFLSSIK

Figure 8:

SEQ ID No 13: DNA sequence of vector pBK1CpnSREst: the expression cassette for the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala::est)

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene)

DNA fragment coding for Cpn10 and Cpn60 is flanked by *SacI* site (pos. 69-75) and *SalI* site (pos. 2138-2143).

In the DNA sequence:

Small letters – the Cpn10-Cpn60 coding fragment,

Capital italics – fragments of vector

Capital letters – fragment coding for EstRB8 from plasmid pBK1Est

Capital bold letters = introduced mutations

ACAGGAAACAGCTATGACCTTGATTACGCCAAGCTCGAAATTAACCTCACTAAAGGGA
*ACAAAAGCTGGAGCTC*cctaacttgggatccaacagttggagagtctagcaaatgaaaatccgtccattacatgatcgtatt
gttgttcgccgtaaagaagaagagaccgcaactgcgggtggtattttaccggcgctgcggcagaaaaacaaatcaaggtgtgt
tatctctgtgggtactggccgtattcttgataatggttcagtgaagcgctggcggtaacgaaggcgatgtgtcgttttgtaaatactc
aggtcaaaatactatcgatcgtggtgaagaattattgatttgaatgaaagtgaatctacggcgttttagaagcttaattattacactca
cttttttatttaacctacaaaatttaaggaaagatcatggctgctaaagacgtattatttgggtgatagcgacgcgcaaaaatgttggttaggt
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caaagatggtgtttctgttcgcgtgaaatcgaattgaaagacaaatcgaaaacatgggcgcacagatggttaagggaagttgcttctca
agccaacgaccaagccggtgacggcacaacgacagcgactgtactagcacaggcgattatcagcgaaggcttgaatctgttgccg
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Replacement Sheet

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acttgctccgtacttcatcaacaaccaagaaaaaatgaccgtagaaatggaaaatccattaattctattggttgataagaaaattgataac
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agatcttgccatcttgacgggtggtcaggttattctgaagagctagggatgtctttagaactgcggatccttcttcttgggtacggcaag
caagggtgttatcgataagaaaacaccgtgattgttgatggcgcaggtactgaagcaagcgtaatactcgtgttgaccagatccgtgct
gaaatcgaaagctcgacttctgattacgacatcgaaaagttacaagaacgcgttgctaagcttgcggggcggttgcgtgattaaggtt
gggtcgggttctgaaatggaatgaaagagaagaaagaccgtgttgacgatgcacttcacgaactcgcgcagcgggtgaagaagggt
gttgttgcgggtggtggtgttgccttgattcgcgcactctctcagtaaccgtgttgggtgataacgaagatcaaaacgtcggtattgcattg
gcacttcgtgcgatggaagctcctatccgtcaaatcgcgggtaacgcaggtgctgCagggGcagCggttgttgataaagtgaatct
ggcacaggtagcttgggtttaaagccagcacaggtgagtatggcgatatgattgcgatgggtattttagaccctgcaaaagtcacgcgtt
catctctacaagccgcggcgctctatcgaggtttgatgatcacaaccgaagccatgggtgcggatgcgcctgttgaagaaggcgctggt
gggtatgcctgatatgggcggcatgggtggaatgggcgggtatgcctggcatgatgtaatcactttgtgattcattgtcctgatctgcttaccg
tGTCGACATATTCAAGATAAAGATGCCTTCACTGACATCAGTCACCAACAATCAAT
CAAACACCAATACCAATCGCAAAAACCTCATAAACTAGCCGATCACCAAAATCCC
AAAAGCGTTCAAAAATGAAACGAGCACGTCACACAAAATCAATTTATACGCTAA
CGAACCAGGTCAAACCTTATCGTTTTTTTGAGCACGTTTGTTCCTACTAATGAAAGAG
AAAAGTCGTTAATTCCTGACTGGCTTTTGGCGTATCCGCACCTTCACATAGAAATTAGT
AATGGCATGCTACTGGCCTTTAAAAAGAATCAGTTAATTGAAGAAACCTCGCTTA
TCTCAGCCATTACCGCTGTAGCCGAATTTGCGCTTATCCTCAGCCATGATTAACT
GACGCCAATTAATATAAGACATACTAATTAATAACTCCCTTAATTGAGAAGAATA
ATGAAAAACACACTCAAATCCTCATCACGTTTTAGTCTGAAACAACTCGGCACCG
GCGCTCTGATTATCTCCAGTTTGTCTTCGGTGGTTGCACCACAACACAAGAT
AATTTATACACAGGGGTATGTCTCTTGCGAGAGACAGCGCTGGCCTAGAAGTTA
AAACAGCCTCTGCCGGTGACGTCAATCTTACTTATATGGAACGCCAAGGCAGTGA
CAAAGATAATGCCGAAAGCGTTATTTTATTACACGGTTTCTCTGCTGATAAAGAT
AACTGGATTCTTTTTACCAAAGAATTCGATGAAAAATATCATGTTATCGCTGTCGA
TTTAGCGGGACATGGCGATTGAGAACAATTATTAACGACTGATTACGGTCTCATA
AAACAAGCCGAGCGTTTAGATATCTTCTTATCTGGCTTAGGGGTAACTCATTTC
CATCGCCGGTAATTCAATGGGGGGGGCTATCAGCGCAATCTACAGTTTGAGTCAC
CCAGAGAAAGTTAAAAGTCTTACATTGATCGATGCAGCAGGTGTCGATGGCGATA
CTGAAAGCGAATACTACAAAGTTTTGGCAGAAGGTAAGAATCCTTTAATTGCAAC
TGATGAAGCAAGTTTTGAATACCGCATGGGTTTCACCATGACTCAGCCTCCTTTCC

TACCTTGGCCACTAAGACCTTCTTTATTACGTAAAACGCTAGCCCGTGCCGAGATC
AATAACAAAATTTTTTCCGATATGCTGAAAACCAAAGAACGTTTAGGAATGACTA
ACTTTC AACAGAAAATTGAAGTGAAAATGGCTCAACATCCATTGCCAACACTGAT
TATGTGGGGCAAAGAAGATCGCGTTCTTGACGTATCCGCAGCAGCGGCCTTCAAA
AAAATAATTCCACAAGCAACTGTTTCATATTTTTCTGAAGTAGGCCACCTACCTAT
GGTAGAAATTCCTAGTGAAAGCGCTAAAGTTTATGAAGAGTTTTTGTCTCTATTA
AATAAGAGCACATAATCATGACTGACTTATAAACAGCCAAGCATTTAAAATGCTT
GGCTGTTTATTTAATGGCCAAATTATTCAACGACCAAGCTCTGCGGTAAAATCG
CAGTGGGTTTCTTGTTTTTCATCAACAGCAACAAACGTGAAATACCCCGTAATCGC
ATTTTTCTGATTATCAAAATACATACTTTCCACCAGCATATTAACCTTCAACTTTTA
AACTCGTCCGCCCTACCTCTATAACACTGGCAGTCAATTTCGACAATGGTACCTGC
GGGAACAGGATGCTTAAAATCGATTTCGATCACTGCTGACGGTTACGATGCTTTGT
CGAGAAAAACGAGTCGCTGCAATAAAAGAAACCTCATCCATCCACTGCATTGCA
GTGCCACCGAATAACGTATCATGATGATTTGTTGTCTCTGGAAATACCGCTTTAGA
AATAGTGGTTTTTGATACGCGCTTTCGCTGCGCAATAATATCTTCTCTGCTAAGAG
TTGCGGATGGCATAACATAAACTCGCTTGATTAAGATTAATAATAAATAGTTAACA
GTATATTGAACTGAGGGTCTGAAGAACTCTAATACCTCTGAAGAACTTTGAGGCC
GCTAGAGAGAAAAGACCAGTGATAATTTTCATCTTGCCATGAGAGCTTATCATG
AAAGCCTGTGCTTAAAATCAATCATTATATTTATTCATCTTTAATTGAAATAATAC
CAATATATTTTCATATATAATTTACACTACCCTTATCTCACTAGACTTCCCGCGCA
TAGGCGCAAACAATCAACGCAAGTTCACAATAAAGCGGTTTCGCTGCAACACATG
CCCTAGCGTCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGCCCCTTTGC
GATTCGTGCAGACGAGCAACAAGCGCTATTAAACTTACCTAAATTTCTAACCACC
ACCATTTGGTTCTTTTCCACAACTCAAAAACTCGTCAAATCCGCTTGCAATTTAA
ACGCGATGACATAGATCTAATCGATTATCAAACCCGCATTCAAGCGCTCATTAAA
AACGCACCACTGGCAAGAAGTTCTACCTGCACTGACCAATATGCAAGCGGCGGC
GGAAGAGCTGCCTTTGATCGATCAAGAAGAAGGGAGCAGCAAAGAGGAAAACA
ATCAAAAAGAGGAGAGCAATCAAATAAAAACGAGTTATTGAGGATTTTAATTTTA
AAACAGGTATATTAATACCCTCTCTCGTAGTAAACAATGACTGTATTTACACAAA
AATAAATAGAGGTATACCATGTCAAACATCTGGTTTGAAGTACCAAAGATTGAAG
TATTAAACCGTCAAATGGAAAATACTGCCTGCAGCAACTTAGGCATTCAAATTAC
AGAAATTGGCGATGATTATATCACTGGCACAATGCCAGCAGATGCACGTACCTTC
CAGCCAATGGGACTGATTCATGGCGGCTCAAATGTATTGCTGGCAGAAACACTGG
GCAGCATGGCAGCTAACTGCTGTATTAATTTGTCTCAAGAATATTGTGTTGGCCA

AGAAATTAACGCCAACACATACGCGGTGTTTCGTTCCGGCATAGTGACTGGCACA
GCAACGCTAGTACACAAAGGAAGAACCTCCCAGATTTGGGAAATTCGCATCGTTA
ACGATCCAAAGAATTCAAAAAGCTTCTCGAGAGTACTTCTAGAGCGGCCGCGGGCCCA
TCGATTTTCCACCCGGGTGGGGTACCAGGTAAGTGTACCCAATTCGCCCTATAGTGAGT
CGTATTACAATTCACTGGCCGTCGTTTTAC

Figure 9:

Amino acid sequence of the stabilized single ring mutant Glu460Ala/Ser462Ala/Val463Ala of Cpn60:

SEQ ID No 14: Cpn10 (nucleotides 458-751 of Figure 10):

MKIRPLHDRVRRKEETATAGGIILPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 15: Cpn60 (nucleotides 458-751 of Figure 10):

MAAKDVLFGDSARAKMLVGVNILDVVRVTLGPKGRNVVIEKSFGAPIITKDGVSV
REIELKDKFENMGAQMVKEVASQANDQAGDGTATVLAQAIIEGLKSVAAGMNP
MDLKRIGDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADETVGRLIAEAMEKVG
KEGVITVEEGKGLEDELVDVEGMQFDRGYLSPYFINNQEKM TVEMENPLILLVDK
DNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVA AVKAPGFGDR
RKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEASV
NTRVDQIRAEIESSTSDYDIEKLQERVA KLAGGVAVIKVGAGSEMEMKEKKDRVDDA
LHATRAAVEEGVVAGGGVALIRALSSVTTVVGDNEDQNVGIALALRAMEAPIRQIAGN
AGAAGA AVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPK VTRSSLQAAASIAGL
MITTEAMVADAPVEEGAGGMPDMGGMGGMGMPGMM

Figure 10:

SEQ ID No 16: DNA sequence of the stabilized single ring mutant

Glu460Ala/Ser462Ala/Val463Ala:

In the DNA sequence:

Small letters – the Cpn10-Cpn60 coding fragment,

Big bold letters = introduced mutations

atcaaaaaatgcagcaaggacagattcctgcccagaattagcagaagggttcttggtagcactggccggcgcttattattaacgccgg
gtttgtcactgatgcgctgggttttacattactcgtccccgcgacgcgtaaagcgttggtccataagggtgattgcattattaccctc
gcatgatgactgcaagcagcttcaagcgacgggtagtttcaggaaggctcgtttaaagatgtacattcgcacactgactcgaaagca
gtcatgaaaaaatcacaattgaaggcgaatatacacaagacgataagtaggtatttttcggctagccgttgaaatcctagtaaaagccc
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ggcgctgcggcagaaaaaccaaatacaaggtgttgttctctgtgggtactggccgtattcttgataatgggtcagtgcaagcgctggc
ggttaacgaaggcgatgtgtcgttttggtaatactcaggtcaaaatactatcgatcgcgatggtgaagaattattgattttgaatga
aagtgatctacggcggtttagaagcttaattattacactcactttttatttaacctacaaaatttaaggaaagatcatggctgctaaagacg
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aattcgaaaacatggcgacagatgggttaagggaagtgcttctcaagccaacgaccaagccggtgacggcacaacgacagcgactg
tactagcacaggcgattatcagcgaaggcttgaatctgttgcggctggcatgaatccaatggatcttaaacgttggtattgataaagctac
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cgatgaaacgggttgctgttaattgctgaagcgatggaaaaagtcggtaaagaaggtgtgattaccgttgagaaggcaaaggccttg
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ctagggatgtcttagaaaactgcggatccttcttgggtacggcaagcaaggtgttatcgataaagaaaaacaccgtgattgttga
tggcgcaggtactgaagcaagcgttaatactcgtgttgaccagatccgtgctgaaatcgaaagctcgactctgattacgacatcgaaaa
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gaccgtgttgacgatgcacttcatgcaactcgcgcagcgggtgaagaaggtgtgttgcgggtggtggtgtgttgccttgattcgcgcactct
cttcagtaaccgtgtgttggtgataacgaagatcaaaacgtcggtattgcattggcacttcgtgcgatggaagctcctatccgtcaaatcgc

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Replacement Sheet

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gctttaacagtaaaataataacgcaacgtagaaacataataagcgtatggcattaatgaagacggctgcatttaattcagatc